REMARKS

Claims

Based on the amendments above, claims 1-47 are pending, claims 1, 35, 36, and 37 are the independent claims, and claims 41-47 are new.

Section 112 Rejections

Claims 25, 27, 29, 32, and 33 have been amended to address the Section 112 rejection. Specifically, they have each been amended to replace "recorded" with "stored" to be consistent with claim 1. Also, claim 30 has been amended to depend from claim 29 to make it different from claim 26.

Section 102 Rejections - U.S. Patent Application Publication 2004/0081621 to Arndt

Claims 1-10, 13-19, 22, 34, and 37-40 stand rejected as allegedly anticipated by U.S. Patent Application Publication 2004/0081621 ("Arndt"). We traverse.

Arndt does not teach or suggest: "illuminating a sample to cause the sample to emit radiation ... spectrally filtering the emitted radiation with each of *a plurality of different spectral weighting functions* ... and processing the ... images [of the spectrally filtered radiation] to construct a deep tissue image of *the sample* in which signal from the other compounds is reduced relative to signal from the target compound," as claimed in independent claim 1 and similarly claimed in independent claim 37 (emphasis added).

To the contrary, Arndt describes using only one spectral filter per sample. Specifically, Arndt describes using a "marker" or a "contrast medium" for fluorescence imaging of a given sample, and a system having multiple spectral filters to provide a specific spectral filter for each such marker, to thereby accommodate multiple samples with different markers. For example, Arndt states:

> Before the actual imaging step ... the mouse ... was administered a contrast medium. This is a specific substance, a so-called "metabolic marker"... [P0047]

> The various markers have different optical properties such as excitation and emission wavelengths, and must therefore be handled differently. [P0048]
>
> By plugging in different filter arrangements 17, it is therefore possible to change easily from an examination with one contrast medium to an examination with another contrast medium. [P0057]
>
> The individual spectral filter combinations are prepared for mutually different contrast media, each of the spectral filter combinations comprising ... a luminescence filter for filtering out wavelengths above the expected emission maximum of the luminescent light emitted by the contrast medium. [P0034]
>
> Imaging experiments with different markers can therefore easily be prepared by exchanging the filters. [P0069]

Thus, Arndt administers a marker/contrast medium to a sample and uses a corresponding a spectral filter. For a subsequent experiment using a new marker/contrast medium, Arndt's system can introduce a different spectral filter corresponding to the new marker/contrast medium.

What Arndt does not teach or suggest, however, is spectrally filtering emitted radiation from a particular sample with *a plurality* of different spectral weighting functions, such as claimed in the rejected independent claims 1 and 37. Moreover, Arndt is completely silent as to processing the multiple images corresponding to the plurality of different spectral weighting functions to construct a deep tissue image of *that sample* in which signal from the other compounds is reduced relative to signal from the target compound, as also claimed in rejected independent claims 1 and 37.

By way of contrast, for example, the specification of the present application explains that the detection level for a component of interest a sample can be greatly improved by analyzing *multiple* spectrally filtered images of that sample. For example, the specification states:

> [I]mages are taken while viewing the emission light from the specimen at a sequence of wavelengths, to develop an image cube with two spatial dimensions and a spectrum at each point. By determining the difference in spectral properties between the desired target compound emission and the unwanted autofluorescence emission, the overall signal is decomposed into components and

> the detection levels for the desired compound emission are greatly improved. [Page 2, lines 6-11.]

Accordingly, we ask the Examiner to withdraw the anticipation rejection of claims 1 and 37, and the rejected claims that depend from them (specifically, dependent claims 2-10, 13-19, 22, 34, and 38-40).

Moreover, while these dependent claims are allowable over Arndt for at least the same reasons as those described above, we further note the following with respect to dependent claims 9 and 10.

The Action incorrectly construes the term "autofluorescence" to be "the reflection of the fluorescent light from an object." To the contrary, autofluorescence refers to background fluorescence produced by endogenous components of the sample. For example, the specification explains how: biological samples exhibit autofluorescence when they are illuminated in the visible range; that autofluorescence can greatly obscure fluorescence from tissues, such as tumors, stained with compounds such as GFP and that such autofluorescence "is also variable from specimen to specimen and can be unpredictable;" and that the overlying dermis of a living sample can, in particular, produce significant autofluorescence." See, e.g., pages 8, 9, and 13 of the application.)

This is also consistent with how the term "autofluorescence" is generally used in the art. For example, "Nature Protocols" defines autofluorescence as:

> The fluorescence from endogenous cell constituents such as NADH, riboflavin and flavin coenzymes, which can contribute to background levels during cell imaging. (http://www.nature.com/focus/cellbioimaging/glossary/index.html, copy attached)

Similarly, Wikipedia describes "autofluorescence" as follows:

> **Autofluorescence** is the fluorescence of substances within an organism. Autofluorescence can be problematic in fluorescence microscopy. In most fluorescence microscopy, fluorescent stains (such as fluorescently-labeled antibodies) are applied to samples to stain specific structures. Autofluorescence interferes with detection of the resulting specific fluorescent signals, especially when the signals of interest are very dim — it causes structures other than those of interest to become visible. Depending upon the shape of the structures of

> interest and the other structures, it may not be obvious that this has occurred. In some microscopes (mainly confocal microscopes), it is possible to make use of different lifetime of the excited states of the added fluorescent markers and the endogenous molecules to exclude most of the autofluorescence. (http://en.wikipedia.org/wiki/Autofluorescence, copy attached)

Indeed, this problem described in the Wikipedia passage, that of structures of interest not being visible because of autofluorescence, is a problem addressed by certain embodiments of the present invention. See, for example, the specification at page 8, line 3, through page 13, line 13.

In contrast, Arndt does not even mention the problem of autofluorescence, let alone provide a solution for the problem. This is yet another reason why the rejection of claims 9 and 10 should be withdrawn.

Section 102 Rejection – U.S. Patent No. 6,891,613 to Wolleschensky

Claims 35 and 36 stand rejected as allegedly anticipated by U.S. Patent No. 6,891,613 ("Wolleschensky"). We traverse.

Each claim requires that the images that are processed are of spectrally filtered radiation emitted, at least in part, from "deep tissue" in the sample. As explained in the application, an image of fluorescence emitted from a tumor inside a mouse is an example of a deep tissue image. (See, e.g., the application at page 9, line 12 through page 13, line 4). The specification also identifies images of emission from structures lying about 2 mm or more within a living organism, with 300 microns or more of overlying dermis, as further examples of a deep tissue image. (See, e.g., the application at page 13, lines 5-13.)

We do not see where the processing of such deep tissue images is taught or suggested in Wolleschensky. To the contrary, the sections of Wolleschensky cited in the Action seem only to describe "a biological preparation" in which "cell nuclei are clearly distinguishable from … cell skeletons." (See Wolleschensky at col. 10, lines 30-32 and 53-55.)

Accordingly, we ask that the rejection of claims 35 and 36 over Wolleschensky be withdrawn.

Furthermore, we have added new claims 42 and 44 that depend from claims 35 and 36, respectively, and recite that "the emission from the other components of the sample [that produce the images that are processed] comprises autofluorescence from tissue overlying the deep tissue," and also added new claims 43 and 45 that depend from claims 35 and 36, respectively, and recite that "the emission comprises emission from a thickness of tissue at least 2 mm thick."

We submit that these additional limitations further distinguish Wolleschensky because Wolleschensky is directed instead to optical techniques that collect light from only a small slice of the sample, such as confocal or multiphoton microscopy. For example, Wolleschensky describes the following "descanned" and "non-descanned" detection techniques for his laser scanning microscope (LSM):

> In confocal detection (descanned detection) of fluorescent light, the light emitted from the focal plane (specimen) and from the planes located above and below the latter reaches a dichroic beam splitter (MDB) via the scanner. This dichroic beam splitter separates the fluorescent light from the excitation light. The fluorescent light is subsequently focused on a diaphragm (confocal diaphragm/pinhole) located precisely in a plane conjugate to the focal plane. In this way, fluorescent light components outside of the focus are suppressed. (Column 2, lines 41-50.)
>
> When using multi-photon absorption, the excitation of the dye fluorescence is carried out in a small volume at which the excitation intensity is particularly high. This area is only negligibly larger than the detected area when using a confocal arrangement. Accordingly, a confocal diaphragm can be dispensed with and detection can be carried out directly following the objective (non-descanned detection). (Column 2, lines 56-62)

Wolleschensky further reiterates the use of such techniques at col. 6, lines 16-21 and lines 34-37.

Moreover, we can find no mention in Wolleschensky of "autofluorescence," problems arising from autofluorescence, or solutions to such problems. This is not surprising because the optical sectioning techniques used by Wolleschensky suppress any emission from tissue overlying or underlying the slice of interest from contributing to the images being processed.

By way of contrast, for example, the specification at page 13, lines 5-13 of the present application distinguishes such optical sectioning techniques:

> In this example, the invention permitted observation of structures in tissue lying ~2 mm within a living organism, where the overlying dermis is at least 300

microns thick and has significant autofluorescence. The invention has also been used to image structures at differing depths in other specimens, including non-mammalian specimens such as zebrafish. In the latter, the specimen is physically thinner, but once again there is the problem of autofluorescence arising from other layers in the specimen, which confounds the detection of target compounds in the interior of the specimen. While there are optical techniques for depth sectioning, such as confocal microscopy, the present invention provides a simple and practical alternative.

In other words, embodiments disclosed in the application use algorithmic processing techniques to suppress the effects of autofluorescence, instead of requiring a more complicated optical apparatus, such as a confocal microscope, to optically suppress emission, if any, from overlying or underlying layers.

Accordingly, we asked that dependent claims 42-45 be allowed.

Section 103 Rejections

Dependent claims 11, 12, 20, 21, and 23-33 stand rejected as allegedly obvious over Arndt in view of one or more secondary references. We submit that these dependent claims are allowable for at least the same reasons as those set forth above for claim 1, from which these claims depend. Accordingly, we ask the Examiner to withdraw the rejection.

Below we also provide additional reasons as to why dependent claims 24-27, 29, 32, and 33, in particular, are allowable.

These claims stand rejected as allegedly obvious over Arndt in view of U.S. Patent No. 6,051,835 ("Pettipiece"). The action concedes that the limitations of these claims are not disclosed in Arndt, but argues that they are disclosed in Pettipiece and that it would have been obvious to modify Arndt in view of Pettipiece "in order to efficiently distinguish the dyes used to identify the target within the object for constructing the image" and "in order to develop an image that shows the target compounds distinguished from the other parts of the supporting tissue." (Action at pages 11-12.) This is incorrect.

For example, claims 25, 29, and 32 each require constructing the claimed deep tissue image based on: i) the stored images of the spectrally filtered radiation emitted from the sample;

and ii) the emission spectrum for the target compound, or the emission spectrum for each of one or more of the other compounds in the sample, or both. This is not what is being described in Pettipiece.

To the contrary, Pettipiece describes using an interferometric technique to spectrally resolve the light collected at each pixel. See, for example, Pettipiece at col. 2, lines 39-43, and col. 5, lines 20-25, 39-50, and 65-67. Accordingly, Pettipiece does measure spectral data from his sample. But, he does not describe processing that spectral data based on the individual emission spectrum of one or more of the components that may be present in his sample, let alone processing that data to construct "a deep tissue image of the sample in which signal from the other compounds is reduced relative to signal from the target compound," as required by the claims.

There is also no disclosure in Pettipiece of the subject matter of claim 24, which recites "processing the stored images comprises constructing the deep tissue image based on a weighted superposition of signals in the stored images." To the contrary, Pettipiece simply states:

> Once the spectral data for each pixel of array 215 has been determined, processor 10 can be used to generate a variety of useful images on screen 111. (Col. 5, lines 65-67.)

We can find no mention in Pettipiece of the claimed "weighted superposition," nor do the sections of the Pettipiece cited in the Action mention this.

Similarly, we can find no mention in Pettipiece of the calculated "remainder spectrum" recited in claims 26 and 30. By way of contrast, the specification of the present application at page 15, line 21 through page 16, line 21, describes the calculated remainder spectrum and explains how it can be used to construct the deep tissue image, even when the pure emission spectrum of one or more of the components in the sample is unknown. We can find no discussion of such a technique in Pettipiece.

Accordingly, we ask the Examiner to withdraw the rejection of claims 24-27, 29, 32, and 33. In the event the Examiner maintains the rejection, we respectfully ask the Examiner to explicitly identify where in Pettipiece he finds the claimed subject matter.

Conclusion

In view of the above, Applicants ask that the Application be allowed.

Canceled claims have been canceled without prejudice or disclaimer.

Any circumstance in which Applicants have: (a) addressed certain comments of the Examiner does not mean that Applicants concede other comments of the Examiner; (b) made arguments for the patentability of some claims does not mean that there are not other good reasons for patentability of those claims and other claims; or (c) amended or canceled a claim does not mean that Applicants concede any of the Examiner's positions with respect to that claim or other claims.

Please charge the excess claim fee in the amount of $350.00 to deposit account 06-1050, referencing 1259-034001. Please apply any other charges or credits to deposit account 06-1050, referencing 12259-034001.

Respectfully submitted,

Date: 1/29/07

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Autofluorescence

From Wikipedia, the free encyclopedia

Autofluorescence is the fluorescence of substances within an organism.

Autofluorescence can be problematic in fluorescence microscopy. In most fluorescence microscopy, fluorescent stains (such as fluorescently-labeled antibodies) are applied to samples to stain specific structures. Autofluorescence interferes with detection of the resulting specific fluorescent signals, especially when the signals of interest are very dim — it causes structures other than those of interest to become visible. Depending upon the shape of the structures of interest and the other structures, it may not be obvious that this has occurred. In some microscopes (mainly confocal microscopes), it is possible to make use of different lifetime of the excited states of the added fluorescent markers and the endogenous molecules to exclude most of the autofluorescence.

In a few cases, autofluorescence may actually illuminate the structures of interest, or serve as a useful diagnostic indicator.

Examples

Without labelling, these substances show fluorescence. Because of scattering, it is better to use the nonlinear two photon excited fluorescence microscopy.

- tryptophan
- indolamine
- fibrillin
- indolamine dimer
- collagen
- NADH (reduced form only)
- elastin
- lipofuscin
- indolamine trimer
- flavin

Categories: Microscopy | Biochemistry stubs

imaging in cell biology

WEB FOCUS imaging in cell biology

glossary

Browse: Anisotropic-diffusion filter | AOTF | Atomic-force microscope | Attenuation | Autofluorescence | Caged compounds | Chromophores | Correlative microscopy | CryoEM | Cryofixation | Fluorescence correlation spectroscopy | Fluorescence polarization | Fluorescence quantum yield | Fluoronanogold | Freeze substitution | Gene gun | High-pressure freezing | ImmunoEM | Laser-scanning microscopy | Magnetoencephalography | Magnetosomes | Microscopic magnetic resonance imaging | Molar extinction coefficient | Multi-photon microscopy | Nanoparticles | Nanoprobe scanning | Optical coherence tomography | Optical tweezers | Pattern photobleaching | Phototoxicity | Positron emission tomography | Projection | Scanning near-field optical microscopy | Segmentation | Single-pair fluorescence resonance energy transfer | Single-photon emission computed tomography | Spectroscopic or chemical-shift imaging | Superparamagnetic magnetite nanoparticles | Surface reconstruction | Tapping mode | Tokuyasu cryosectioning technique | Tomogram | Total internal reflection | Ultrasmall gold | Volume rendering | Zoom

ANISOTROPIC-DIFFUSION FILTER

An image processing method for reduction of shot noise without degradation of an image.

AOTF

Acousto-optic tunable filters (AOTFs) are birefringent crystals bonded to a piezoelectric transducer. Application of a radio-frequency signal to the transducer generates acoustic waves in the crystal that alter the index of diffraction of the crystal and result in diffraction of certain wavelengths of light. A change in the frequency or amplitude of the applied radio signal leads to a change in the wavelength and intensity of the diffracted light. As the radio signal can be altered rapidly, the intensity and wavelength selection are rapidly altered accordingly.

ATOMIC-FORCE MICROSCOPE

A type of scanning-probe microscope, in which a fine needle attached to the tip of a soft cantilever scans the surface of a specimen. The shape and physical properties of the surface can be detected from the bending of the cantilever. This type of microscope can be used to manipulate single molecules.

ATTENUATION

Blocking or modulation of the excitation light intensity can be accomplished with a series of filters that transmit increasing percentages of the incident light or with an acousto-optic tunable filter (see AOTF).

AUTOFLUORESCENCE

The fluorescence from endogenous cell constituents such as NADH, riboflavin and flavin coenzymes, which can contribute to background levels during cell imaging.

CAGED COMPOUNDS

Caged fluorophores are fluorophores that have been chemically modified with a caging group that quenches their fluorescence until a brief pulse (usually <100 ms) of ~350-nm light breaks the photolabile bond connecting the fluorophore and the caging group.

CHROMOPHORES

Groups with characteristic optical absorptions. They usually contain alternating single and double bonds.

CORRELATIVE MICROSCOPY

A combination of methodologies that allows a fluorescent signal that is first seen in living cells to be processed at any given time for immunoelectron microscopy, so that the fine structure and molecular make-up of the carrier that elicited the signal can be revealed.

CRYOEM

A technique by which, using a special cryoholder, cryofixed biological samples are directly imaged in the transmission electron microscope under low-dose conditions and at low temperature (at least -170 °C). The sample can be either a frozen layer of suspension that contains many isolated macromolecules for single-particle reconstruction techniques, or a thin (100–500 nm) section.

CRYOFIXATION

The rapid freezing of small samples such that cellular components can be immobilized in milliseconds.

FLUORESCENCE CORRELATION SPECTROSCOPY

A microspectroscopy technique, in which fluorescence-intensity fluctuations in a small focal volume (~1 μm^3) are measured to enable the number, size and movement of fluorescent molecules to be determined at the single-molecule level.

FLUORESCENCE POLARIZATION

The absorption of a fluorophore depends on the polarization angle of the excitation light, and fluorescence emission is also polarized. Fluorescence polarization is used to detect the direction and rotation of molecule movement.

FLUORESCENCE QUANTUM YIELD

The ratio of photons emitted to photons absorbed. The fluorescence brightness of a species is proportional to the product of its molar extinction coefficient and fluorescence quantum yield. See also the definition for molar extinction coefficient.

FLUORONANOGOLD

Fluorescent complexes of small gold clusters that are tagged with an antibody or Fab fragment.

FREEZE SUBSTITUTION

A procedure in which, at low temperature, the cellular water of, for example, cryofixed material is replaced by a series of organic solvents, including chemical fixative. As a final step, embedding media are applied (mostly epoxy resins) that, after polymerization, allow further processing of the sample.

GENE GUN

Ballistic particle-mediated gene transfer. Complementary DNA molecules are adsorbed to gold particles and shot by a pressure gas jet into tissues or culture cells.

HIGH-PRESSURE FREEZING

A cryofixation procedure that uses high pressure to freeze samples efficiently and to minimize the formation of damaging ice crystals.

IMMUNOEM

The detection of identified proteins by electron microscopy, which makes use of specific antibodies that are tagged with a marker, usually colloidal gold, for visualization in the electron microscope.

LASER-SCANNING MICROSCOPY

A technique for generating an image with a microscope pixel by pixel, which involves sequentially scanning a laser beam through the focal plane of the objective lens and collecting the fluorescence with a single detector.

MAGNETOENCEPHALOGRAPHY

An *in vivo* imaging technique that detects tiny magnetic fields generated by electrical current loops, which are typically due to brain activity.

MAGNETOSOMES

Magnetic particles that are created within organelles.

MICROSCOPIC MAGNETIC RESONANCE IMAGING

A variant of clinical magnetic resonance imaging, which has been adapted for non-invasive studies of small samples that range in size from rats to frog embryos. Typical spatial resolutions are in the range of tens to hundreds of micrometres.

MOLAR EXTINCTION COEFFICIENT

The molar extinction coefficient (ε) of a species is defined by the equation $A = \varepsilon bc$, where A is the absorbance of the solution, b is the path length, and c is the concentration of the species. The fluorescence brightness of a species is proportional to the product of its molar extinction coefficient and fluorescence quantum yield. Highly fluorescent molecules such as rhodamine have high values of both molar extinction coefficient and fluorescence quantum yield. See also the definition for fluorescence quantum yield.

MULTI-PHOTON MICROSCOPY

A microscopy technique that uses simultaneous absorption of two or more photons of a long wavelength to excite fluorophores that are normally excited by a single photon of shorter wavelength. The use of the longer excitation light reduces photodamage and allows excitation of fluorophores located deep within thick samples.

NANOPARTICLES

Particles with controlled dimensions on the order of nanometres. Examples

include colloidal gold, magnetite particles, and luminescent semiconductor aggregates that are also known as 'quantum dots'.

NANOPROBE SCANNING

A family of imaging techniques in which the interaction of a sharp, nanometre-sized tip with a specimen is measured as the tip is mechanically scanned over the specimen surface.

OPTICAL COHERENCE TOMOGRAPHY

An *in vivo* imaging technique that sends out femtosecond infrared pulses and uses optical interference to sense reflections from tissue inhomogeneities.

OPTICAL TWEEZERS

A laser light that is focused by the objective lens of a microscope can be used to trap a plastic bead with a diameter of 0.1–10 μm. The trapped bead can be used as a 'handle' to allow the manipulation of molecules.

PATTERN PHOTOBLEACHING

Marking geometrical patterns on biological structures that have amorphous shapes, in order to measure structural dynamics.

PHOTOTOXICITY

Damage of the living specimen following excessive illumination on fluorescence microscopes. Phototoxicity increases with shorter wavelengths.

POSITRON EMISSION TOMOGRAPHY

An *in vivo* imaging technique that detects the location of positron-emitting isotopes by virtue of the pair of γ rays that are emitted when the positrons encounter electrons.

PROJECTION

Reduction of dimensionality. For example, a 3D Image (x/y/z) can be projected into the x/y plane by assigning the maximum intensity that can be found along the z axis at each x/y position to a single projection image. This generates a 2D maximum intensity projection.

SCANNING NEAR-FIELD OPTICAL MICROSCOPY

An evanescent field from a small aperture at the tip of an optical fibre is used to excite fluorophores in this type of microscope. The fibre is scanned over the specimen to obtain 2D images. The optical resolution, which is limited to the size of the aperture, is very high (~30 nm).

SEGMENTATION

The identification of objects above background noise using image-processing methods. Can be achieved by detecting either object boundaries (contour-orientated segmentation) or whole objects (region-based segmentation).

SINGLE-PAIR FLUORESCENCE RESONANCE ENERGY TRANSFER

Fluorescence resonance energy transfer (FRET) that occurs from a single donor fluorophore to a single acceptor molecule is called single-pair FRET.

SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY

A nuclear-medicine imaging technique that reconstructs the distribution of

a γ-emitting radionuclide tracer in a subject in 2D or 3D.

SPECTROSCOPIC OR CHEMICAL-SHIFT IMAGING

A variant of magnetic resonance imaging that generates individual nuclear magnetic resonance spectra from a grid of subvolumes in an object. In addition to the more conventional water and lipid magnetic resonance images, analysis of the resulting spectra allows maps of specific metabolites to be reconstructed.

SUPERPARAMAGNETIC MAGNETITE NANOPARTICLES

Nanometre-sized particles of magnetite (Fe_3O_4), which locally amplify an external magnetic field, but are too small to maintain their own field in the absence of an external field.

SURFACE RECONSTRUCTION

Visualization of surface polygons from arbitrary angles in a graphical viewer. Requires previous object identification usIng segmentation techniques.

TAPPING MODE

A form of atomic-force microscopy in which the tIp is vibrated perpendicular to the specimen plane to avoid gouging the specimen as the tip is scanned laterally.

TOKUYASU CRYOSECTIONING TECHNIQUE

An immunoelectron-microscopy procedure In which cells are chemically fixed and infiltrated with cryoprotectant before they are frozen by being plunged into lIquid nitrogen. Sectioning is carried out at a low temperature, after which the frozen sections are transferred to room temperature, thawed, subjected to immunolabelling and, only then, embedded in a thin layer of support and contrasting film.

TOMOGRAM

A 3D image, which is computed from a series of images that are acquired by tilting a 200–500-nm specimen from, for example, −70° to +70° using 1° increments.

TOTAL INTERNAL REFLECTION

When a light beam strikes the interface of two media of different diffractive indices, at an angle beyond the critical angle, all of the light energy is reflected back into the incident medium. In this situation, an 'evanescent field' develops at the interface with an energy that decreases exponentially into the medium with the lower refractive index. This allows selective excitation of fluorophores located within ~100 nm of the interface.

ULTRASMALL GOLD

Gold clusters of 0. 8–1. 4 nm that are often attached to an antibody or Fab fragment. The clusters are visualized in the electron microscope by using a silver- or gold-based size-enhancement procedure.

VOLUME RENDERING

Computer visualization of 3D images from arbitrary angles without explicit definition of surface geometry.

ZOOM

The optical zoom lens system assists the objective in image magnification. It determines the size of the scan region and the apparent magnification of

the image. Pixels in a zoom-1 image have areas twice the width and length of those in a zoom-2 image. Increasing the zoom magnification leads to increased irradiation time per unit area and therefore to increased photobleaching.